Cell cycle: **How, when and why cells get rid of cyclin A**

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Sequences outside the 'destruction box' direct the degradation of cyclin A to completion before the metaphase–anaphase transition; cyclin A that escapes timely degradation can block the metaphase–anaphase transition, impede anaphase and telophase, and impair a cell's ability to arrest in G1 of the next cell cycle.

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Activity of the cyclin-dependent kinase Cdk1 drives a cell into mitosis, and its inactivation allows the cell to resume interphase. What happens in between is a remarkable sequence of events. Chromosomes condense, congress into a metaphase plate, and achieve bipolar attachment to the mitotic spindle. Sister chromosomes then disjoin with phenomenal speed and are pulled to opposite poles. Only after that process is complete does cytokinesis occur to split the cytoplasm, and the separated sister chromosomes, into two daughter cells. The consequences of deviating from this order — for example, segregating sisters before complete disjunction, or executing cytokinesis without prior chromosome segregation — would be disastrous. How does the cell orchestrate mitotic events with precise sequential order in each and every cell division?

The invariant sequence in mitotic chromosome dynamics is accompanied by the sequential degradation of specific proteins. An 'E3' ubiquitin-conjugating enzyme complex, the anaphase promoting complex or cyclosome (APC/C), in association with an activator, Cdc20/Fizzy, targets proteins for degradation during mitosis (reviewed in [1]). Another version of APC/C, in association with a different activator, Cdh1/Fizzy-related, functions in later mitotic stages in some cell types (see [2], for example). The experimental stabilization of individual proteolytic targets results in arrest of mitosis at specific stages (detailed below). Thus, understanding what drives sequential protein degradation during mitosis is key to understanding how the invariant order of mitotic events is achieved.

An important group of proteins targeted for mitotic destruction are the cyclins that bind and activate the cyclin-dependent kinases (Cdks). Of these, cyclin A is arguably the most enigmatic. In contrast to the mitotic cyclins which activate Cdk1 to initiate mitosis, or the S phase cyclins which activate Cdk2 to initiate S phase, cyclin A can bind both Cdk1 and Cdk2 [3], and functions in both S phase and in mitosis (see [4,5], for example). Furthermore, regulation of cyclin A proteolysis appears different from that of other mitotic cyclins. Despite being substrates of the same E3 enzyme, the degradation of cyclin A concludes before that of B-type cyclins in diverse species. Moreover, activation of the spindle checkpoint (see below) stabilizes B cyclins, but not cyclin A, further attesting to differential regulation [6,7]. How differential regulation of cyclin degradation is achieved, and what purpose it serves, have been questions of much interest and investigation. Five recent papers [8–12] — two published recently in *Curent Biology* [10,11] — add live cell imaging to the usual repertoire of genetic and cell biological tools to further our understanding of these issues.

How: 'D' is for 'dispensable'

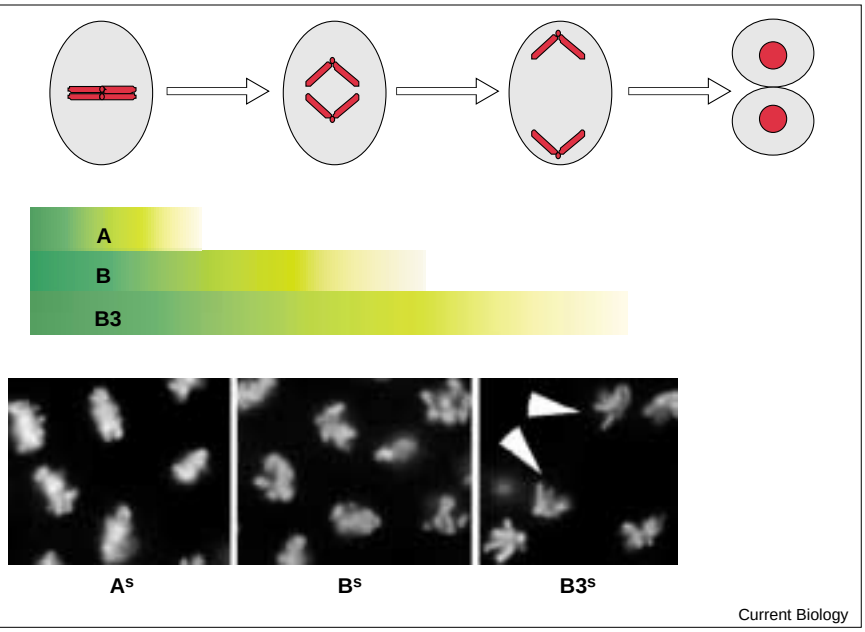
Amino-terminal sequences direct the degradation of mitotic cyclins. A consensus sequence, known as the 'destruction' or 'D' box, is necessary for degradation of B cyclins, and can direct mitotic degradation of unrelated proteins [13]. Both *Drosophila* cyclin A and the human somatic equivalent cyclin A1 have putative D boxes. Surprisingly, mutant forms of the protein that lack these sequences are still degraded with wild-type kinetics [8–12]. It turns out that amino-terminal sequences that do not show a clear consensus direct the degradation of cyclin A in humans and *Drosophila*. So-called 'KEN-box' sequences, which target proteins for degradation via Cdh1–APC, also contribute to degradation of *Drosophila* cyclin A, although the role of Fizzy-related, the fly Cdh1 homolog, remains to be investigated. The degradation signals in human and *Drosophila* cyclin A are thus complex and differ from those of B-type cyclins.

When: cyclin A and the spindle checkpoint

The different sequence requirements for degradation of cyclin A and the B-type cyclins likely mean that these cyclins interact with the APC in different ways. This difference could explain why cyclin A, and not cyclin B, is degraded when the spindle checkpoint is active. This checkpoint is active when kinetochores are not bound to spindle microtubules, either before the establishment of stable bipolar spindle attachment during normal mitosis or because of damage to the spindle by drugs (reviewed in [14,15]). Under these conditions, a complex of proteins that include MAD2 binds and disrupts the ability of Cdc20–APC to ubiquitinate B-type cyclins. It is possible that the MAD2–Cdc20–APC complex is still able to interact with and ubiquitinate cyclin A.

The different fates of cyclins A and B in the presence of an active spindle checkpoint may contribute to the temporal

Figure 1



Sequential events during mitosis and the effects of stable forms of the mitotic cyclins [11,16]. Cyclin A degradation normally concludes in metaphase, and stable cyclin A (A^S) blocks anaphase and subsequent mitotic phases. Cyclin B degradation concludes in anaphase, and stable cyclin B (B^S) blocks anaphase B and subsequent stages. Cyclin B3 degradation concludes in late anaphase, and stable cyclin B3 ($B3^S$) blocks telophase and the return to interphase. Endogenous cyclins are degraded in cells arrested with stable cyclins; thus each cyclin, with associated Cdk1, may be capable of blocking the mitotic events that normally follow its disappearance. If so, sequential cyclin degradation could drive the sequence of mitotic events. Chromosomes are shown as red ovals in the schematics. Bars represent approximate duration of persistence of each cyclin. Photographs of stable cyclin arrest phenotypes show chromosomes stained for a mitotic specific epitope on histone H3, and are reprinted with permission from [24]. Arrowheads show a pair of segregated chromosome sets within a single cell.

difference in their degradation during normal mitosis. By quantitative imaging in live human cells, Geley *et al.* [8] and den Elzen *et al.* [9] found that the cyclin A level, detected via a green fluorescent protein (GFP) tag, begins to decline shortly after nuclear envelope breakdown, suggesting that the APC is active earlier in mitosis than previously thought. In contrast, the cyclin B1 level does not start to decline until metaphase [8]. In human cells with a disabled spindle checkpoint, the cyclin B1 level declines immediately after nuclear envelope breakdown [8]. Thus, an active spindle checkpoint during early mitosis appears to delay the degradation of cyclin B until the checkpoint becomes inactivated, whereas degradation of cyclin A may not be affected by the checkpoint. These features may explain the temporal separation in disappearance of cyclins A and B.

Why: stable cyclin A affects many mitotic transitions

To determine the importance of cyclin A degradation, all five groups [8–12] examined the consequence of stabilizing cyclin A. Overexpression of stable human cyclin A was found to slow or block progression through anaphase and telophase, an effect also seen in *Drosophila* studies [8–12]. In trying to understand why the effects of stable cyclin A are seen at stages during which cyclin A is normally absent, it is worth noting that stable cyclins appear to compete poorly with endogenous cyclins, at least in *Drosophila* [10–12]. Kaspar *et al.* [10] propose that stable cyclin A may not become functional until degradation of endogenous cyclin A liberates the limiting component(s). These could include Cdk1 or substrates that need to form stable complexes with cyclin–Cdks. These notions led Parry and O'Farrell [11] to examine the second mitosis after expression of stable cyclins in *Drosophila* embryos. They found that stable cyclin A effectively blocked the metaphase–anaphase transition and sister chromosome disjunction for this mitosis. This effect is consistent with results from previous studies on fixed *Drosophila* embryos [16], and with the recent finding that stable cyclin A slows progression through metaphase in *Drosophila* even when at competitive disadvantage [12].

In contrast to overexpression, expression of stable cyclin A at endogenous levels had little effect on mitosis [12]. Thus, it could be argued that contribution of cyclin A to timing of metaphase–anaphase transition is minimal. It is worth recalling, however, the case of *Drosophila* securin, Pim. Securins stabilize the cohesion between sister chromosomes until the metaphase–anaphase transition, when they are degraded [17]. A stable version of Pim can block chromosome disjunction, but only when overexpressed; expression of stable Pim at endogenous levels was found to have little effect [18]. A more likely scenario, therefore, is one where cyclin A and Pim, together with additional inputs such as the Cdk inhibitor Rux [19], make partial but significant contributions to collectively time metaphase–anaphase transition.

The budding yeast securin, Pds1, can block not only sister chromosome separation but also subsequent mitotic events, including exit into the next interphase [20,21]. This seems a particularly useful ability when cells with DNA damage arrest in metaphase, presumably to allow time to repair the DNA damage, and a concomitant inhibition of mitotic exit is imperative for ensuring cell viability. In

contrast, Pim and human securin can block only sister chromosome separation and not later mitotic events, even when stable forms of the protein are overexpressed [18,22], making these poor choices for mediators of checkpoint arrest — if cells relied on these proteins, they would exit mitosis without disjoining sister chromosomes. Cyclin A, which as the new data indicate can, at sufficient levels, block both sister chromosome separation and subsequent mitotic phases, would seem to be a good mediator of checkpoint arrest and does indeed seem to play such a role [23].

Sequential cyclin degradation and the temporal order of mitotic phases

Previous studies of fixed embryos [16] and the new work with live cells [11] show a remarkable correspondence between the arrest phenotype and the mitotic stage at which degradation of each cyclin is completed (Figure 1). Because endogenous cyclins are degraded in cells arrested with stable cyclins, cyclins A, B and B3 (with associated Cdk1) appear capable of blocking a nested set of mitotic events. These observations led to a model in which the sequential degradation of mitotic cyclins dictates the temporal order of steps in chromosome dynamics. This attractive model is likely to be a partial picture at best; multiple inputs likely time each transition, as discussed above for the metaphase–anaphase transition. Moreover, cells arrested with stable cyclin B3 were found to undergo cytokinesis [11], indicating that we have much to learn about how the timing of this crucial cytoplasmic division is regulated and coordinated with nuclear division.

Another compelling reason for removing cyclin A

Low levels of stable cyclin A in *Drosophila* embryos were observed not to impede mitotic progression, and cells expressing this protein exited mitosis [12]. Remarkably, though, Jacobs *et al.* [12] found that these cells entered an ectopic S phase — and then an extra mitosis — instead of entering the prolonged G1 arrest typical of their developmental stage. As *Drosophila* cyclin A binds only to Cdk1, and not Cdk2, the ectopic S phases were presumably triggered by the presence of (stable) cyclin A–Cdk1 complexes that persisted through the previous mitosis. Incidentally, this S phase role of cyclin A had previously prevented a clear demonstration of its mitotic role. This is because the inability of cyclin A mutants to enter mitosis could have been the result of a failure to complete S phase, which then activates a checkpoint that prevents mitosis. By analyzing double mutants of cyclin A and a gene required to activate the DNA replication checkpoint, Jacobs *et al.* [12] have provided clear evidence that cyclin A has an essential role in entry into mitosis in *Drosophila*.

The multiple abilities of cyclin A — to drive the entry into S phase and mitosis, and to block progression though much of mitosis — means that its activity must be strictly regulated, perhaps by multiple mechanisms. Trying to understand regulatory controls that act on cyclin A and its targets should prove to be formidable but rewarding challenges.

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